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Effects of a Contaminated Dredged Material on Laboratory Populations of the Tubicolous Amphipod 'Ampelisca abdita'

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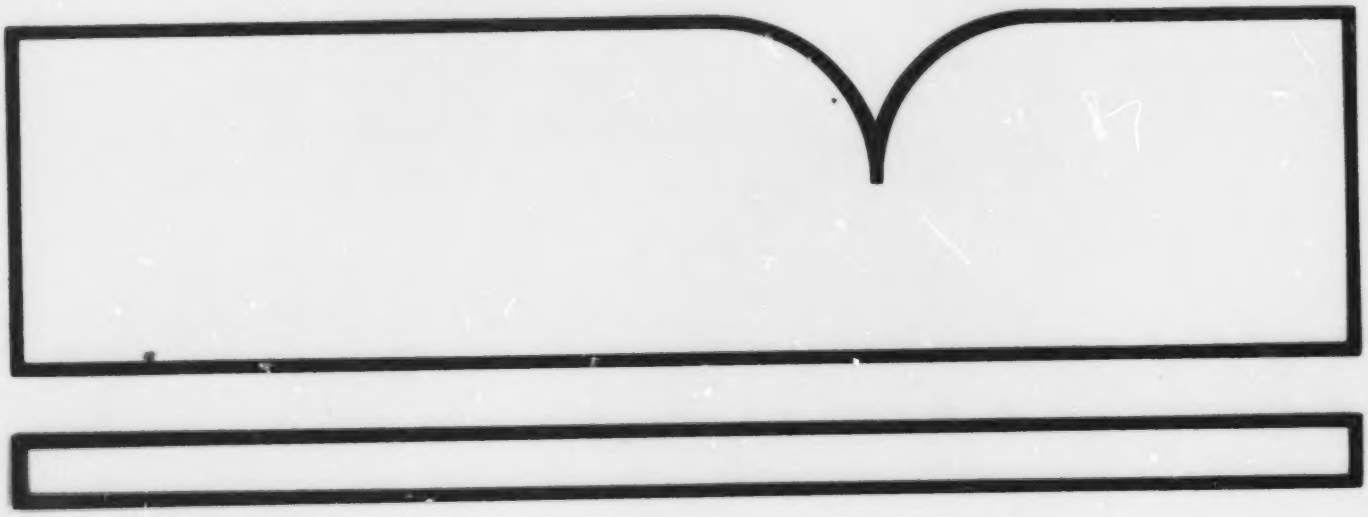

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The Effects of a Contaminated Dredged Material on Laboratory Populations of the Tubicolous Amphipod *Ampelisca abdita*

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ABSTRACT: Short-term and full life cycle toxicity tests have been conducted with *Ampelisca abdita* as part of the joint COE-EPA field Verification Program (FVP). These studies comprised a portion of the effects assessment component of a risk analysis describing the hazards associated with aquatic disposal of dredged materials from Black Rock Harbor (BRH), Connecticut. The specific objective of these toxicity tests was to document the sensitivity of *Ampelisca*'s chronic endpoints to a range of contaminated suspended particulate concentrations. In long-term exposures of 56 days, amphipod growth and intrinsic rate of population growth, r, were impaired at all BRH sediment exposures. In all cases, the reduction in population growth rate was a function of slower growth of females, causing a longer time to maturity, which was coupled with a reduced egg production at maturity because the females were smaller.

KEY WORDS: amphipods, *Ampelisca abdita*, chronic toxicity, contaminated sediments, survival, growth, reproduction, demography

It is well recognized that acute toxicity data generated from 96-h exposure periods, while useful, are insufficient to identify acceptable toxicant concentrations that do not adversely affect growth and reproduction [*1-3*]. This limitation has been addressed by the development of chronic toxicity tests designed to assess pollutant effects on survival, growth, and reproduction over long periods of exposure, often an entire life cycle. Unfortunately, these chronic effects are measured at the individual organism level of biological organization and are not coupled in a predictive manner to population responses [*4,5*].

The National Research Council's report, *Testing for the Effects of Chemicals on Ecosystems* [*6*], recommends that appropriate and relevant decisions regarding the release of potentially toxic chemicals into the environment be based upon a hierarchy of biological tests, with those at the level of populations and communities being particularly crucial to such an assessment. The intrinsic rate of population growth, r, integrates age-specific survivorship and all facets of reproduction, including age at reproductive maturity, number of offspring per brood, frequency of reproduction, and reproductive longevity. Using this demographic parameter, the growth rate and reproductive potential of a population under both contaminant and environmental stress can be determined. The life history of the benthic amphipod *Ampelisca abdita* lends itself to this type of analysis in that all aspects of the reproductive process can be readily discerned

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and quantified, providing a biological and ecologically defensible basis for regulatory decisions.

Ampelisca abdita is a tube-dwelling amphipod which constructs a soft, upright, membranous tube, 3 to 4 cm long, in surface sediments. It ranges from Maine to Louisiana from the intertidal zone to depths of 60 m [7]. This amphipod is a particle feeder, ingesting either surface-deposited particles or particles in suspension. Fertilization occurs in the water column, and they brood their young after mating. The egg-bearing females return to the bottom, and, after a brood period of approximately two weeks, young are released into the surrounding sediment. They are reproductively active at about 5 mm and grow to a maximum length of 7 to 9 mm [8]. Available information indicates that each female reproduces only once.

The present study was part of the joint Army Corps of Engineers—U.S. Environmental Protection Agency Field Verification Program (FVP), whose objective was to field verify existing test methodologies for predicting the environmental consequences of dredged material disposal under aquatic, intertidal, and upland conditions. The Environmental Research Laboratory—Narragansett was responsible for conducting the aquatic portion of the FVP with a primary goal to evaluate the risk of the open water disposal of contaminated dredged materials from Black Rock Harbor, (BRH), Connecticut. The aquatic portion of the FVP assessed the relative sensitivity and utility of various biological endpoints in both the laboratory and in the field following the disposal of BRH sediments [9]. The chronic experiments with *Ampelisca* were designed to simulate the fringe areas of a dredged material mound where suspended sediments are the primary route of exposure. Acute studies were conducted to simulate the short-term nature of a disposal operation and near-field plume transport. Long-term, chronic exposures examined the biological responses to mixtures of contaminated and noncontaminated sediments in the suspended phase with the sediments reduced or fully oxidized. The latter condition represented the potential effects of the resuspension and transport of aged dredged material. The objectives of the experiments described in this paper were (1) to assess the feasibility of conducting full life cycle tests using *A. abdita* and (2) to determine the relative sensitivity of the various biological responses.

Methods

Sediment Collection, Dosing, and Exposure

Two sediment types were utilized to conduct these laboratory tests. A reference sediment (REF) was collected from the South Reference site in Long Island Sound (40°7.95′N and 72°52.7′W) by Smith-MacIntyre grap (0.1 m^2), press sieved through a 2-mm sieve, and stored at 4°C until used. Prior to dredging, a contaminated sediment was collected from Black Rock Harbor, Connecticut (41°9′N and 73°13′W) with a gravity box corer (0.1 m^2) to a depth of 1.21 m, thoroughly mixed, press sieved through a 2-mm sieve, and refrigerated (4°C) until used. Details of sediment collection and storage procedures may be found in Rogerson et al. [10]. These authors also describe in detail the chemical properties of these two sediments. In all experiments, sediments were allowed to reach test temperature and homogenized prior to use.

Two identical sediment-dosing systems were constructed to provide either BRH or REF material as suspended sediment to the exposure system [9]. Each dosing system (Fig. 1) consisted of a conical-shaped slurry reservoir placed in a chilled fiberglass chamber, a diaphragm pump, a 4-L separatory funnel, and several return loops that directed the particulate slurry through microprocessor-controlled dosing valves. The slurry reservoirs (40 cm diameter by 55 cm high) contained 40 L of slurry composed of 37.7 L of filtered seawater and 2.3 L of either BRH or REF sediment. The fiberglass chamber (94 by 61 by 79 cm high) was maintained between 4° and 10°C using an externally chilled water source to minimize microbial degradation during the

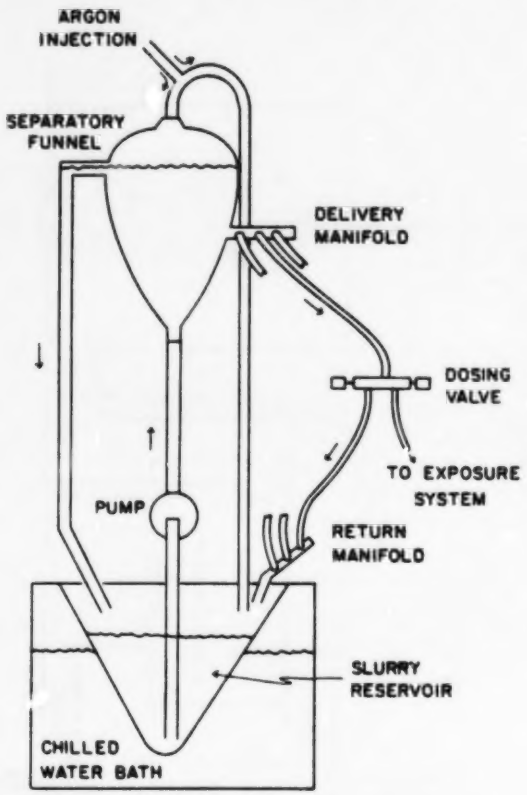

FIG. 1—*Suspended sediment dosing system.*

test. Upon dosing, the slurries were mixed with Narragansett Bay seawater filtered (to 15 μm) through sand filters to provide the desired concentrations for the toxicity tests.

In the first set of experiments, sediments were maintained under reduced (anaerobic) conditions by providing argon gas at the rate of 200 mL/min to the reservoir and separatory funnel. In the second experimental series, the sediments were fully oxidized prior to use in the dosing and exposure systems by transferring 2 L of sediment to an inverted polycarbonate carboy, diluting it to 19 L with filtered natural seawater at room temperature, and aerating for three to four days. Chemical oxygen demand measurements indicated that this time period was sufficient to satisfy the immediate oxygen demand of the sediments.

For the acute range-finding tests, the slurry was delivered to a mixing chamber (glass 4 L reagent bottle) in the amphipod exposure system, where it was initially diluted with seawater at a preset flow rate (right side of Fig. 2). The diluted suspension then passed from the bottom of the bottle to a distribution chamber (17 cm diameter by 9 cm high) fitted with a standpipe to maintain a constant water level. The suspension flowed via a siphon, at a flow rate controlled by head pressure, to a collection funnel which distributed the material to the exposure system.

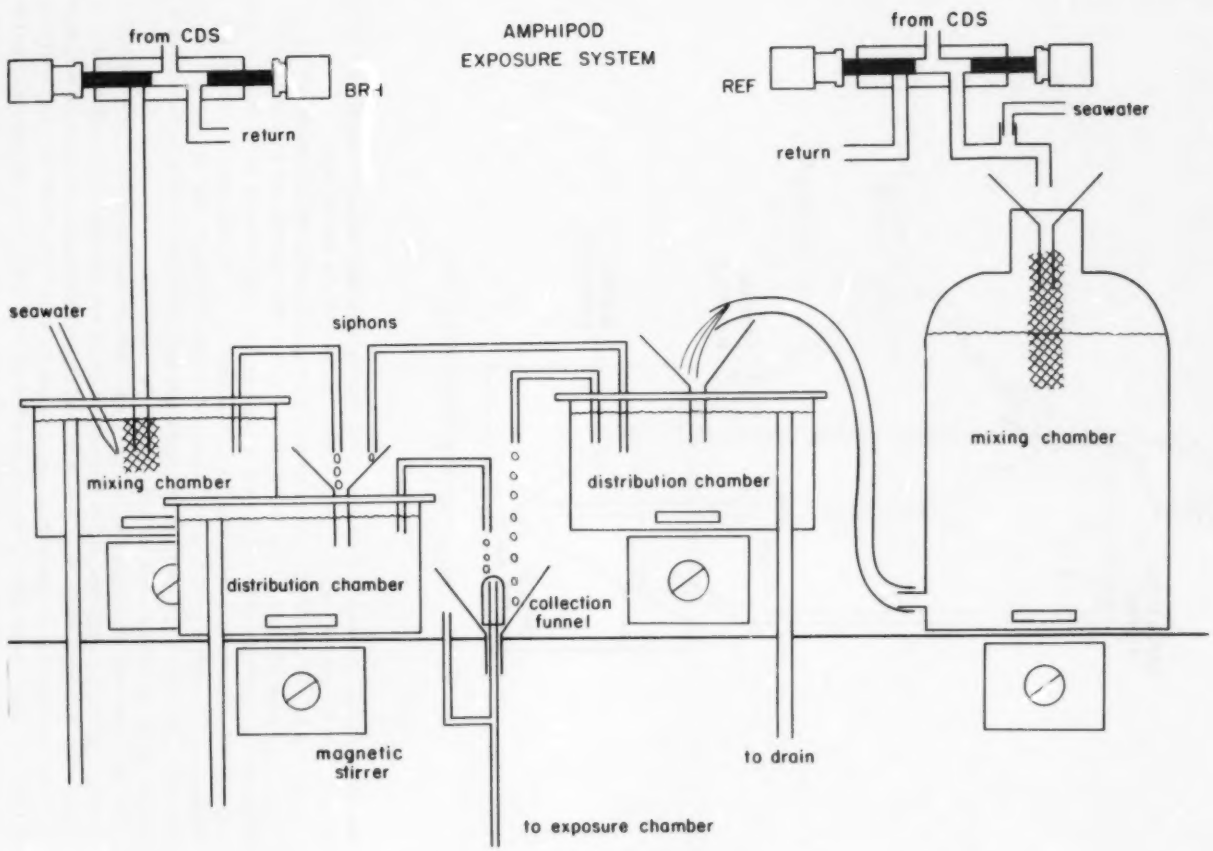

FIG. 2—*Suspended sediment proportional diluter system for short- and long-term tests with* A. abdita.

Each collection funnel was fitted with an umbrella siphon acting as a flow accelerator; these fed the exposure chambers through a polypropylene tube and tee to split the flow to each of two replicates. The mixing and distribution chambers were set up in duplicate, where one mixing chamber was dosed with the BRH sediment while the other was fed filtered seawater only.

For the longer-term tests, a constant suspended particulate density of 50 mg/L was desired, where BRH sediments were diluted with REF sediment instead of with seawater. To achieve this exposure, REF sediment was dosed to one mixing chamber and BRH sediment to the other so that there was an equal particle concentration in each distribution chamber. The limiting factor of this dosing design is the minimum flow rate, 10 mL/min, that can readily be attained from the siphons. Since the chronic tests required much lower BRH sediment concentrations, a modified design was implemented (left side of Fig. 2). The BRH concentration in the distribution chamber was set to 50% by feeding that chamber 50 mL/min of REF and 50 mL/min of BRH at equal particle densities.

The exposure chambers for all suspended particulate tests were glass gallon jars (Fig. 3) filled with 0.75 L of REF sediment (5 cm deep). The suspension was maintained in the gallon jar by aeration and was removed from the jar by a siphon which collected material from just above the sediment surface. The effluent entered a water trap with a screened standpipe which permitted the monitoring of swimming activity and the presence of mating adults in the water column.

The flow rate to each gallon jar was 40 mL/min for the acute tests and was reduced to 20 mL/min for the long-term tests to reduce the turnover rate and, hence, maximize the concentration of food supply. In the latter tests, the diatom *Phaeodactylum tricornutum* was cultured [*11*] and delivered to each gallon jar at 1 mL/min using a Harvard peristaltic pump. The total suspended solids concentration in the exposure system was monitored as total dry weight in mg/L. One-hundred-millilitre samples were taken twice weekly from each gallon jar using a free-standing siphon to minimize disturbance. Temperature and salinity were measured daily. In all experiments, temperature was maintained at 20 ± 1°C. Salinity varied between 28 and 32 parts per thousand. Exposure system flow rates were checked biweekly and adjusted when necessary. The photoperiod for all suspended phase tests was 14-h light and 10-h dark.

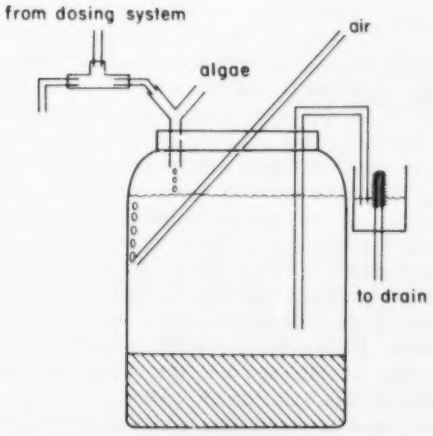

FIG. 3—*Chronic exposure chamber for suspended sediment toxicity tests with* A. abdita.

Experimental Procedure

Ampelisca were collected from tidal flats in the Pettaquamscutt River, a small estuary flowing into Narragansett Bay, Rhode Island. Bulk sediment containing the amphipods was immediately transported to the laboratory and was sieved through a 0.5-mm screen. The *Ampelisca* were collected by flotation from the air-water interface. When collection temperatures were lower than the test temperature, amphipods were acclimated at 1°C/day up to 20°C. During this acclimation, these animals were fed, ad libidum, laboratory-cultured *Phaeodactylum tricornutum*. At the initiation of a test, animals were sieved from the holding sediment and randomly distributed to the appropriate exposure chamber. The solid phase treatment for all suspended phase tests was REF sediment. Two 96-h tests were conducted with 200, 100, 50, and 25 mg/L of BRH suspended sediment and a seawater control. In the first range-find test with reduced sediments, 50 subadult amphipods were exposed in one chamber per treatment; in the second test, there were 25 subadult amphipods in each of two replicates per treatment. These 96-h range-find tests provided data on the acute responses to the raw dredged material.

Since the chronic tests were designed to use a mixture of REF and BRH sediments, a second set of acute tests was also conducted in order to set the BRH concentrations for the long-term exposures. These tests were of intermediate duration and lasted 18 days using reduced sediments and 10 days with oxidized BRH sediments. There were two replicates per treatment with 100 and 50 subadult *A. abdita* per replicate in the reduced and oxidized tests, respectively. Each of these tests, as well as each of the chronic tests, was conducted at a nominal constant suspended solids concentration of 50 mg/L.

The mortality data from the two acute test series were used to set concentrations for the two chronic tests. The concentrations of BRH sediment were 10 and 5% in the first chronic test with reduced sediments and 4 and 2% in the second test with oxidized sediments. Fifteen egg-bearing females were added to each of four replicates per treatment. The size and number of eggs of 30 females were determined prior to the start of the test. Two replicates were sieved after 32 and 28 days of exposure, and the remaining two replicates were harvested after 58 and 56 days of exposure for reduced and oxidized sediments, respectively.

The overflow water trap was checked daily, and the amphipods were enumerated and sexed when possible. Live *A. abdita* were returned to the exposure chambers. At the end of each test, all containers were sieved and the amphipods were enumerated. Any animals not accounted for were considered dead.

In addition, animals recovered from the chronic test were sexed and then measured using a computerized digitizer and camera lucida. The data collected at the 32 and 28-day sampling of the chronic experiments included survival, mean size, and proportion of amphipods that were mature. In addition, the number of eggs held by each ovigerous female was enumerated and the developmental stage determined. These same data were collected at the termination of the test, as was the total number of young amphipods produced per chamber. When the number of young per chamber exceeded 100, these young were subsampled for size measurements using a Folsom plankton splitter, which randomly splits the sample in halves. A minimum sample size of 30 was arrived at by examining the size variability found in previous tests and by determining the minimum sample size necessary to observe a treatment effect at the 5% probability level.

The intrinsic rate of growth, r, is typically calculated by constructing life tables [*12*] to determine age specific survivorship and fecundity. The Euler equation [*13*] is then used to calculate r by successive approximation. This technique has been previously applied [*9*] to these data to demonstrate the utility of this population statistic, but because of the tenuous nature of the underlying assumptions, a similar and more direct approach is presented here.

The intrinsic rate of growth, r, is a direct function of the difference between per capita birth and death rates. *Ampelisca* birth rates were calculated as the number of young produced by the F_1 generation, those young found at the end of the experiment, divided by the predicted number

of adults in the F_1 generation. This value is then divided by the time interval in days over which their young were produced. The death rates were calculated as the number of surviving adults in the F_1 generation, those remaining at the end of the experiment, by the predicted number of young released by the initially introduced ovigerous females. This value was divided by the time interval in days over which these deaths occurred. For both of these determinations, it was assumed that the F_1 generation was released from the brood sac between Days 0 and 14 for the birth rate calculation and that no deaths occurred subsequent to the release and establishment of juvenile *Ampleisca* in their tubes. The final and most basic assumption in this analysis is that these populations are experiencing exponential growth.

Acute toxicity data were analyzed using the probit method [*14*]. Analysis of variance was used to analyze survival, growth, and reproductive data from all tests. Significant treatment differences were identified from Dunnett's or Tukey-Kramer's pairwise comparison tests [*15*]. Reproductive data for *A. abdita* were evaluated by analysis of covariance to account for differences in female size.

Results and Discussion

Short-Term Exposures

Exposure concentrations and resultant *Ampelisca* mortality data for the 96-h range-find tests with reduced and oxidized BRH sediments are shown in Table 1. The measured suspended sediment concentrations were close to the nominal concentrations for the lower treatments, while the measured concentrations for the two highest treatments (100 and 200 mg/L) had greater variability. *Ampelisca* mortalities exhibited a consistent dose response in both tests,

TABLE 1—Ampelisca abdita *mortalities and suspended sediment concentrations ($\bar{x}$ + SD) for BRH sediment exposures of four days.*

Nominal Concentration, mg/L	Measured Concentration BRH, mg/L, $\bar{x}$ + SD (n)	Mortality, %
	REDUCED EXPOSURES[a]	
0	...	4
25	34 ± 11 (2)	20
50	55 ± 9 (2)	20
100	92 ± 45 (2)	50
200	239 ± 30 (2)	88
	OXIDIZED EXPOSURES[b]	
0	...	6
25	31 ± 9 (3)	26
50	51 ± 13 (3)	30
100	76 ± 4 (2)	48
200	167 ± 38 (3)	74

NOTE: $\bar{x}$ = mean; s = standard deviation; n = number of replicates.

[a] LC_{50} = 91 mg/L (95% confidence interval = 76 to 111). Initial size of *A. abdita* ($\bar{x} \pm s$), 3.39 ± 0.89 mm (n = 25).

[b] LC_{50} = 80 mg/L (95% confidence interval = 64 to 106 mg/L). Initial size of *A. abdita* ($\bar{x} \pm s$), 6.40 ± 0.89 mm (n = 50).

yielding LC_{50}s of 91 and 80 mg/L BRH sediment for the reduced and oxidized exposures, respectively.

Based on the 96-h mortality data, exposures of a longer duration were designed to examine the acute responses when BRH sediments were diluted with REF sediments. Since moderate toxicities occurred at approximately 30 mg/L (Table 1), dilution concentrations were chosen to provide BRH levels at or below this concentration. In the reduced sediment test, amphipods were exposed for 18 days to 75, 50, 25, and 0% (control) BRH sediment at a constant suspended solids concentration of 50 mg/L. Significant mortalities were observed at all concentrations (Table 2), even in the 25% BRH treatment (12 to 13 mg/L BRH sediment). Another experiment was conducted with oxidized sediments exposing *Ampelisca* for ten days at 50, 25, 12.5, and 0% (control) BRH sediment. The toxicities observed in this test were slightly lower than those seen in the reduced exposures and were likely the result of a shorter exposure period.

Long-Term Exposures

Mortality—Suspended sediment concentrations for the reduced sediment exposures had a range of mean concentrations of 40 to 50 mg/L across all replicates, and there were no significant treatment or replicate differences. The total suspended solids concentrations in the oxidized exposures were slightly higher, ranging from 50 to 57 mg/L, and similarly showed no significant treatment differences. The calculated BRH concentration for each of the treatments in these two tests and their respective chronic mortalities are shown in Table 3.

TABLE 2—*Suspended sediment concentrations and* Ampelisca *mortality for an 18-day exposure to reduced and a 10-day exposure to oxidized BRH sediments.*

Treatment	Replicate Number	Total Suspended Sediment Concentration, $\bar{x} \pm s$ (n)	Calculated BRH Concentration, mg/L	Mortality, %
		REDUCED—18 DAY[a]		
REF control	1	56 ± 28 (6)	0	14
	2	53 ± 36 (6)	0	9
25% BRH	1	50 ± 22 (6)	13	90
	2	51 ± 23 (6)	13	98
50% BRH	1	51 ± 12 (6)	25	100
	2	56 ± 9 (6)	28	100
75% BRH	1	56 ± 6 (6)	42	100
	2	54 ± 7 (6)	40	100
		OXIDIZED—10 DAY[b]		
REF control	1	56 ± 4 (5)	0	0
	2	53 ± 3 (5)	0	2
12.5% BRH	1	61 ± 8 (5)	8	12
	2	59 ± 6 (5)	7	14
25% BRH	1	51 ± 5 (5)	13	70
	2	51 ± 8 (5)	13	74
50% BRH	1	41 ± 9 (5)	21	88
	2	44 ± 11 (5)	22	72

[a]The number of amphipods per replicate is 100. Initial size of *A. abdita*, ($\bar{x} \pm s$), 3.11 ± 0.48 mm (n = 91).

[b]The number of amphipods per replicate is 50. Initial size of *A. abdita*, ($\bar{x} \pm s$), 4.74 ± 0.43 mm (n = 50).

TABLE 3—*Estimated* Ampelisca *adult mortality in long-term exposures to reduced and oxidized BRH suspended sediments.*

Treatment	Calculated BRH Sediment Concentration, mg/L	Number of Ovigerous Females Introduced	Harvested/ Number Predicted	Estimated % Mortality
		Day 32—Reduced		
REF control		30	495/532	7.0
5% BRH	2.0	30	585/532	0.0
10% BRH	4.5	30	417/532	21.6
		Day 56—Reduced		
REF		30	286/532	46.2
5% BRH	2.3	30	191/532	64.1
10% BRH	5.0	30	384/532	27.8
		Day 28—Oxidized		
REF control		28	304/320	5.0
2% BRH	1.0	28	332/320	0.0
4% BRH	2.0	29	285/331	13.9
		Day 56—Oxidized		
REF control		30	123/342	64.0
2% BRH	1.0	29	172/331	48.0
4% BRH	2.0	30	115/342	66.4

Survival in each exposure was estimated by comparing the number of *Ampelisca* harvested with the estimated initial number of young, assuming 100% survival of all eggs of the ovigerous females that were introduced into the containers at Day 0. For reduced exposures, these females had a mean egg number of 17.7 eggs ($s = 6.73$) to yield 532 initial young. As shown in Table 3, survival in the control and 5% exposures was better than in 10% BRH at Day 32. The 95% confidence interval around the mean egg number per female was 15.2 to 20.2 eggs yielding 454 and 606 initial young; the 10% BRH falls significantly outside this range. The mortality threshold appears to occur at 4.5 mg/L BRH sediment based upon these 32-day data.

For the oxidized exposures the initial sample of ovigerous females had an egg number of 11.4 ($s = 4.98$) yielding 342 young per 30 females. The number of amphipods harvested on Days 28 and 56 are also shown in Table 3. The predicted number of juveniles was adjusted to 160 for some containers because one ovigerous female died in each of those replicates on the first day. The 95% confidence limits are 9.5 to 13.3 eggs/female resulting in 266 to 372 offspring for 28 females, 276 to 386 offspring for 29 females, and 285 to 399 for 30 females. For the 28-day exposure period there were no significant mortalities.

Mortalities in the adult population at the 56-day harvest ranged from 27.8 to 64.1% for the reduced exposures and from 48.0 to 66.4% for the oxidized exposures. These mortalities are not considered to be due to BRH sediment exposure. They represent a six to eight-week life span in laboratory populations with a significant contribution to the mortalities being males, who die subsequent to mating. Of the 138 mortalities observed in the overflow cups across all treatments, 76% were adult males.

The mortalities observed in the 10% BRH treatment of the reduced exposures are noteworthy. The presence of significant mortalities at Day 32 was the reason for lowering the nominal concentrations in the second test with oxidized sediments. One would expect that this effect

would be compounded after a 56-day exposure, however the converse was true; this treatment period exhibited the lowest mortality. As will be shown below, this result is not inconsistent and actually reflects an effect of BRH sediment on *Ampelisca*'s growth and the eventual structure of the population.

Population Structure and Individual Growth—Individuals can be sexed and classified into stages of sexual maturity based on morphological characteristics. Morphologically, adult males (M) are the most distinctive with a carinate urosome for swimming, which facilitates mating in the water column. They do not molt again because they die after mating. Whether they mate with more than one female is unknown.

Female *A. abdita* can be divided into four progressively distinct groups. The earliest stage, after sexual differentiation, is here termed developing female (FDV). This stage is distinguished by the presence of a brood plate (oostegite) on the interior of each of the first five coxal appendages. As the female grows, the brood plate increases in size and eggs begin to develop in the oviduct, which is dorsal to the digestive tract and can be seen through the exoskeleton. This stage is termed developing egg female (FE). After a series of molts the brood plates develop long setae and the eggs are deposited through a gonopore into a fully developed brood pouch. This stage is termed ovigerous female (FOV). Presumably, mating occurs at this time. Since all eggs in a brood pouch are in the same stage of development, all eggs in the oviduct are assumed to be deposited at once. After releasing their young, the females do not immediately die but remain in the population for some undetermined time before death. These females may have eggs in the oviduct, but do not have eggs in the brood pouch, at the same time retaining the setose oostegites. This stage is termed spent females (FS).

There is one other group, termed undifferentiated (UD). These include juveniles and undifferentiated subadult females and males. In most cases where this group was encountered, the animals were larger than 4.0 mm. These animals are probably subadult males since females can be distinguished at a smaller size when the brood plates first develop.

The percentage of amphipods in each population class is shown in Table 4. In the first experiment with reduced sediments, the 32-day harvest revealed that the majority of the population was as yet immature (undifferentiated), constituting 45% or greater of the population in all these treatments. The control and 5% BRH exposures did have higher proportions of adult males and females. This trend became more obvious in the 58-day samples where these two treatments contained much higher proportions of ovigerous and spent females and females with eggs in the oviduct. Conversely, the 10% BRH treatment was dominated by developing females and undifferentiated amphipods. These results signify a retardation in the development and maturation at the highest BRH sediment exposures. Because the number of mature males present in the 10% BRH treatment was reduced at 58 days, and because natural mortality of mature males was a major contribution to total mortality at this exposure period, mortality in the 10% BRH treatment was less than that in the other treatments, as noted previously.

This same pattern was repeated in the second experiment using oxidized sediments, although the differences were not as dramatic. At 28 days, the undifferentiated group dominated the population structure. There were no apparent treatment differences in the rate of maturity. After 56 days the population had matured, as expected, with most of the females being ovigerous. There was a trend for there to be fewer immature animals (UD, FDV) in the control treatment, which also produced many more spent females. These data indicate that the control exposure was producing a slightly more reproductively active population.

Examination of the mean lengths of each of these population classes helps to explain the differences observed in the population structure. These data are presented in Table 5. At Day 32, in the reduced sediment test, there were significant treatment differences in the mean lengths of the undifferentiated group, with the control amphipods largest and the 10% BRH exposed animals the smallest. The developing females and those with eggs in the oviduct were also smaller as a result of this exposure. At 58 days, the 10% BRH exposed amphipods were

TABLE 4—*Percent of* A. abdita *in each population category for long-term exposures to reduced and oxidized BRH suspended sediments.*

Treatment (BRH Sediment)	Category[a]					
	UD	FDV	FE	FOV	FS	M
			Day 32—Reduced			
REF control	50.5	22.3	14.2	5.2	...	7.8
5% BRH (2.0 mg/L)	45.3	23.1	16.6	4.8	...	10.3
10% BRH (4.5 mg/L)	63.8	25.2	10.1	0.7	...	0.2
			Day 58—Reduced			
REF control	17.5	14.7	14.0	29.7	9.4	14.7
5% BRH (2.3 mg/L)	10.5	6.3	13.6	43.5	16.8	9.4
10% BRH (5.0 mg/L)	43.8	32.8	7.8	6.5	0.8	8.3
			Day 28—Oxidized			
REF control	69.5	21.2	6.4	2.9	0	0
2% BRH (1.0 mg/L)	68.3	24.6	5.3	1.9	0	0
4% BRH (2.0 mg/L)	80.7	16.1	0.3	2.6	0	0.4
			Day 56—Oxidized			
REF control	8.1	4.1	1.6	38.3	31.0	17.0
2% BRH (1.0 mg/L)	16.7	17.0	12.4	26.8	14.5	12.8
4% BRH (2.0 mg/L)	14.6	12.2	6.7	40.2	11.6	14.9

[a]See text for designation of categories.

smaller than controls in all population classes. The developing egg females and ovigerous females in the 5% BRH treatment were also smaller than those in the control treatment. These data clearly show that slower growth of exposed amphipods accounts for the changes in the population structure and overall maturity.

In the oxidized sediment experiment, the growth pattern described above was repeated. At Day 28, the undifferentiated group and the developing females were smallest at the 4% BRH sediment exposure. At Day 56 the ovigerous and spent females were also smallest at this exposure level. The adult males in the two BRH exposures were larger than those in the controls. Again, there were not as many significant size differences in this second experiment. This lack of differences was probably due to the lower exposure concentrations used in this test. When comparing the common concentration in the reduced (5% BRH) and oxidized (4% BRH) experiments, where exposures were approximately 2.0 mg/L BRH sediment, the size data and significant differences for ovigerous females are surprisingly consistent. The lengths of these females were significantly different from those in controls in both exposures, resulting from the slower growth that would have been predicted from the 28-day data.

The population proportion and size data presented here are consistent with estimates of growth and time to first reproduction described by Mills [*8*] for field populations of *A. abdita* from Barnstable Harbor, Massachusetts. He found that for summer populations, at approximately 20°C, growth was essentially linear and that newly released juveniles grew to breeding adults in 40 to 80 days. Adult females grew to a mean size of 5.5 to 6.0 mm. The *Ampelisca* in these two experiments grew from hatching at approximately 1.5 mm to 6 to 7 mm in a minimum of about 50 days. This would be a minimum time span since all juveniles were not released on Day 1 but rather over a two-week time span.

To summarize the population structure and growth data, there appears to be an adverse

TABLE 5—*Mean length (mm) of* A. abdita *in long-term exposures to reduced and oxidized BRH suspended sediments.*

Treatment, (BRH Sediment)	Sex Category					
	UD	FDV	FE	FOV	FS	M
			DAY 32—REDUCED			
REF control	5.74	5.29[a]	6.37	6.14	...	5.50
5% BRH (2.0 mg/L)	5.47	5.22	6.39	6.34	...	5.59
10% BRH (4.5 mg/L)	4.42	4.67	6.04	6.75	...	5.25
			DAY 58—REDUCED			
REF control	7.33	7.18	7.58	6.89	NM	6.40
5% BRH (2.3 mg/L)	7.23	6.88	6.99	6.25	NM	6.24
10% BRH (5.0 mg/L)	6.15	5.80	6.69	6.12	NM	5.83
			DAY 28—OXIDIZED			
REF control	3.77	4.07	4.62	6.59	...	...
2% BRH (1.0 mg/L)	3.77	4.11	5.03	6.98	...	...
4% BRH (2.0 mg/L)	3.21	3.78	4.41	6.44	...	...
			DAY 56—OXIDIZED			
REF control	6.69	5.69	6.13	6.77	6.74	5.83
2% BRH (1.0 mg/L)	6.60	6.28	6.95	6.88	6.94	6.17
4% BRH (2.0 mg/L)	6.32	5.95	7.13	6.21	6.09	6.16

NOTE: NM = not measured.
[a]Means connected by the same line are not significantly different from each other at $P < 0.05$.

impact on growth, which, although small, does have an effect on maturation. This effect on maturation was more evident at 28 or 32 days than it was at the 56 or 58-day sampling. As will be shown below, the impact on growth may be accompanied by more subtle effects of exposure to dredged material on egg production and juvenile survivability.

Reproduction—Fecundity, the number of eggs/female, in *A. abdita* is a function of female size, with larger females producing larger broods [*8*]. Treatment effects were assessed using analysis of covariance (ANCOVA), with egg number as the dependent variable and female size as the covariate. Once the quantitative relationship between egg number and female size is determined, the differences in egg number/female can be analyzed by statistically adjusting the fecundity estimates based on the regression relationship.

The raw and size-adjusted fecundity estimates for the 58- and 56-day harvests in the two sediment exposures are shown in Table 6. The number of eggs/female in the reduced sediment exposures showed no significant treatment effects when adjusted for female size. The number of young produced, however, were dramatically different among treatments. There were no young observed at the 10% BRH sediment exposure even though there were spent females present (Table 6). This would indicate some problem with egg viability or hatching and survival success. Young were observed in the 5% BRH treatment, but there densities were much lower than those in the control treatment.

TABLE 6--*Reproductive characteristics of* Ampelisca *exposed to reduced and oxidized Black Rock Harbor suspended sediments for 58 and 56 days, respectively.*

Treatment	Female Size, mm	No. Eggs/Female		Young Produced
		Raw	Size Adjusted	
		REDUCED		
REF	6.89	13.6	12.4	1212
5% BRH (2.3 mg/L)	6.25[a]	12.3	13.5	318
10% BRH (5.0 mg/L)	6.12	9.2	10.9	0
		OXIDIZED		
REF	6.77	15.8	15.4	1012
2% BRH (1.0 mg/L)	6.88	15.8	15.0	117
4% BRH (2.0 mg/L)	6.21	10.8	12.0	122

[a]Means connected by the same line are not significantly different.

Brood size of *A. abdita* was also reported by Mills and, more recently, by Nelson [*16*] for North Carolina populations. Mills found 26.0 eggs per female for a mean female size of 5.4 mm, while Nelson observed 13.7 eggs per female for females 5.3 mm in length. The egg numbers reported here are within the range of those found previously, as are the sizes of ovigerous females.

In the oxidized sediment exposures, there were significant treatment effects on fecundity. In the 4% BRH treatment, the ovigerous females were not only smaller (Table 5), and, thus, produced fewer eggs, but also had lower fecundities when compared with similar-sized animals in the other two treatments.

The effect of oxidized dredged material exposure on fecundity and growth is further borne out by examining the number of young produced (Table 6). There were over eight times the total number of young produced in the controls as compared with the 4% BRH treatment. Unexpectedly, however, the productivity of the 2% BRH treatment was also lowered, even though fecundity was equal to that of the control. This again indicates possible effects on either hatching success or juvenile survival, neither of which were directly measured in this experiment.

The mean sizes of these juveniles from the oxidized sediment exposures were analyzed by ANOVA, which shows the controls to be significantly larger ($P < 0.05$) than the other two treatments (Control = 2.29 mm, 2% BRH = 1.65 mm, 4% BRH = 1.72 mm). The control juveniles were either released sooner or were growing faster. In either case, the assumption is that, with continued exposure, they would have matured and reproduced sooner than juveniles in the two BRH treatments. These size data and the productivity results are the first evidence in these experiments of detrimental impacts at the lowest BRH exposure levels.

These results, in conjunction with the previous information on population structure and individual growth, support the conclusion that the impact of dredged material on reproductive output tends to be compounded with each generation. In this particular case, the exposed parental populations grew slower and exhibited delayed maturation. The mature females were not only smaller but also produced fewer eggs, resulting in fewer young in the next generation. Because these filial young hatch at a later date, time to first reproduction, as compared with control populations, is already adversely affected. The implications of effects on growth and time to first reproduction are, that in temperate (seasonal) environments, survival of late brooders may be severely impacted by being out of synchrony with the normal water temperatures and food supplies that are necessary for naturally growing populations.

Population Growth—The utility of measuring the various population parameters described above is that they can be used in a predictive nature. A means to accomplish this is to make projections or estimates of population growth, stability, or both, using population response parameters such as intrinsic rate of growth.

The population responses parameters, birth rate, death rate, and intrinsic rate of growth (r) are shown in Table 7 for reduced and oxidized exposures. Example calculations of birth and death rates for the control treatment in the reduced exposures are as follows. The number of young produced by the F_1 generation was 1212 (Table 6). This value was divided by the predicted number of F_1 adults in this treatment, 532 (Table 3). The quotient was then divided by the number of days over which these young were produced, 49, assuming Day 7 as the release date, to arrive at a birth rate of 0.045 individuals per capita per day. The death rate is the number of *Ampelisca* dying between Days 7 and 56 divided by the predicted number of organisms in that treatment (Table 3), or [(532 − 286)/532]/49. The difference between birth and death rates, r, was less than zero in all BRH sediment exposures, indicating that these populations will eventually become extinct under continued exposure to these sediments. The death rates for all exposures, including the controls, are relatively similar and reflect the fact that these suspended sediment concentrations were neither acutely nor chronically toxic. In fact, the lowest death rate was observed in the highest BRH sediment exposure (10% BRH). As described above, this low death rate is related to slower individual maturation, which delayed reproduction, mating, and senescent death. As can be seen in Table 7, the major factor controlling the intrinsic rate of growth, using this analysis, was the birth rate. This factor was severely depressed in all BRH sediment treatments.

The primary reason for the differences in population responses noted is the much lower reproductive output of the two BRH treatments (Table 6), which is a function of delayed maturation, in combination with lower fecundities in those treatments. As discussed above, the smaller mean size of the progeny at the 56-day harvest would cause even later maturation of those individuals, leading to the potential for continued negative r values.

Conclusions

The biological responses of acute mortality and growth did not differ greatly between the reduced and oxidized sediment exposures. However, amphipod fecundity was affected more dramatically by exposure to oxidized sediments. In both exposures, there were survival problems in the second generation juveniles that were not apparent in the first generation. The

TABLE 7—*Population responses for* A. abdita *exposed to reduced and oxidized BRH suspended sediments for 58 and 56 days, respectively.*

Treatment	Death Rate, individuals/capita/day	Birth Rate, individuals/capita/day	Intrinsic Rate of Growth
	REDUCED		
REF Control	0.009	0.045	0.036
5% BRH, 2.3 mg/L	0.013	0.012	−0.001
10% BRH, 5.0 mg/L	0.005	0.000	−0.005
	OXIDIZED		
REF Control	0.013	0.060	0.047
2% BRH (1.0 mg/L)	0.010	0.007	−0.003
4% BRH (2.0 mg/L)	0.014	0.007	−0.007

results suggest that the long-term exposure of the maturing females to contaminated sediments may affect the viability of developing embryos.

It is clear that the primary biological response to these contaminated sediments is slower growth. Slower growth delayed maturation, increasing the time to first reproduction and decreasing the size of breeding females. The implication to the population is lower numbers of young being produced over a longer time period, therefore forcing the population out of synchrony with natural breeding cycles which are normally temperature and food-source dependent.

Additionally, this research demonstrates the ability to culture *Ampelisca abdita* under laboratory conditions through at least two generations. The population size increased 2.5 to 3.0 times between the interim, 28 and 32-day sampling, to the final harvest at 56 and 58 days. If all ovigerous females were allowed to brood their young to hatching, much larger numbers would be expected after 56 days.

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